Preparative Work on Pepsin-Digested Denatured Collagen

By KIRSTI LAMPIAHO, ANNIKKI KARI, T. HOLLMÉN, J. PIKKARAINEN and E. KULONEN. (*Department of Medical Chemistry, University of Turku, Turku* 3, *Finland*)

Denatured rat-tail tendon collagen is almost instantly degraded with pepsin to a mixture of fragments which remains relatively stable for a few hr. and yields a characteristic starch-gel electrophoretic pattern of about 6–7 bands (Penttinen, Kari & Kulonen, 1966; Lampiaho, Kari, Niinikoski & Kulonen, 1966). In addition there appears some material which does not stain with nigrosine in the gel sheet but can be detected with the ninhydrin and biuret reactions. After a digestion of 24hr. the pattern becomes more complex but the mixture contains still large fragments.

The breakdown-mixture was fractionated by combining gel-filtration on a Sephadex G-200 column which was eluted with dilute acetic acid, column chromatography with carboxymethylcellulose and phosphocellulose (Bornstein & Piez, 1966) and preparative starch-gel electrophoresis (Hollmén & Kulonen, 1966). Some fragments, which resolve in the analytical starch-gel electrophoresis, could not be prepared in pure form in spite of systematic attempts.

Two of the large fragments were characterized further. A fraction, designated α', which migrates in the starch-gel electrophoresis just ahead of the α1-component, has a mol.wt. of 62500 by sedimentation ($S^0_{20,w} = 2{\cdot}68$s) and 60500 by amino acid composition (assuming one histidine and two hydroxylysine residues). Electrophoretic experiments at various gel concentrations and ionic strengths suggest that the α'-fragment is derived from the α1-component. The composition of this fragment corresponded to that of whole collagen, but tyrosine was absent and the ratio of hydroxyproline/proline was 0·84. The α'-fragment could be broken down to three subfractions with cyanogen bromide (Bornstein & Piez, 1965), which agrees with the two methionine residues. Likewise the fragment α' could be broken further with pepsin. Three main subfragments were observed in the starch-gel electrophoresis.

The other purified large fragment, corresponding to the band D in starch-gel electrophoresis (Penttinen, Kari & Kulonen, 1966), was rich in valine, leucine and arginine but poor in glutamic acid and aspartic acid. The ratio of hydroxyproline/proline was 0·97. The molecular weight was 28000 by sedimentation and 29900 assuming one methionine residue in the fragment.

This work was supported by institutional grants from the U.S. Department of Agriculture, Foreign Research and Technical Programs Division, and from the Sigrid Jusélius Foundation.

Bornstein, P. & Piez, K. A. (1965). *Science*, **148**, 1353.
Bornstein, P. & Piez, K. A. (1966). *Biochemistry*, **5**, 3460.
Hollmén, T. & Kulonen, E. (1966). *Analyt. Biochem.* **14**, 455.
Lampiaho, K., Kari, A., Niinikoski, J. & Kulonen, E. (1966). *Acta chem. scand.* **20**, 1446.
Penttinen, R., Kari, A. & Kulonen, E. (1966). *Acta chem. scand.* **20**, 1304.